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Multivariate Signal Processing for Quantitative and Qualitative Analysis of Ion Mobility Spectrometry data, applied to Biomedical Applications and Food Related Applications

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by

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INTRODUCTION

A. Motivation

Nature is always a source of inspiration for science and technology. Step by step industry has adapted normal nature behaviors into applications for enhancing industrial processes or for being used in quality control process. For instance, human panel, which have really developed the sense of smell and taste, have been used in food industry as quality control system (Pedro Santos et al., 2010). Dogs have been also used for detecting explosives or chemical weapons in dangerous places (Oh et al., 2015, Furton and Myers, 2001). Moreover, in the last decades physician would often use the smell sense for diagnosis diseases, for example if the breath of the patient had a sweet flavor, it meant that he or she would have diabetes (Pauling et al., 1971, Teranish.R et al., 1972). Nowadays, volatile organic compounds have become a great interest in medicine for its potential use as diagnosis tool (Baumbach et al., 2005, Buszewski et al., 2007, Horvath et al., 2009, Phillips et al., 1999, Phillips et al., 2003a).

For instance, human panel smells chemical compounds present in samples and a cognitive process processes (identification) the information and resolve it according some references in the brain. In the same way, chemical sensors arrays can be used instead of human panel (olfactory system), and algorithms for classification and identification can replace the cognitive process of the brain (Persaud and Dodd, 1982). Note, both human panel and dogs are able to response in few seconds, thus it is expected that sensor array provide a response in less time with similar or higher reliable results than the nature system.

Furthermore, when a taster establishes the quality of a product, i.e. wine, the olfactory system is actually identifying a pattern from a mixture of chemical compounds and some of them at low concentrations. That means that the instrument must distinguish each compound present in the mixture of a sample with a high sensitivity. Moreover, it should work in real time because nature systems give a response in few seconds. Therefore, the chemical sensors array or any other similar technology need to cover all these features for providing a powerful product to the industry.

In general, chemical sensors are quite sensitive but poorly selective. Chemical sensors usually has a fast response but do not accurately identify the composition of the compounds present in the sample. There are analytical techniques such as Gas Chromatography- Mass Spectrometry which are able to identify each compound present in a sample, but the analysis take at least half an hour. On the contrary, there are analytical techniques such as spectrometers that allows fast response and certain degree of specificity but do not have enough libraries for identifying compounds. Certainly, there is a commitment between sensitivity, specificity, selectivity and fast response that need to be handled in order to precisely mimic the olfactory system.

Despite of the fact there has been remarkable improvements for translating the functionality of the olfactory system into technology, this technology is far from mimic completely the olfactory system above all when the olfactory system is composed of thousands of olfactory receptor neurons of the same type(Firestein, 2001). These huge amount of neurons directly implies to have large sensor array for exactly reproduce the olfactory system. In this context, there have been several studies with large sensor array for reproducing and studying the diversity importance in olfactory system, among of them (Dinatale et al., 1993, Fernandez et al., 2010, Fonollosa et al., 2013, Gutierrez et al., 2011). Even though, there are relevant results from these studies, there is a significant difference between this large arrays and the physiologic function of the olfactory system. There are seriously problems that have been actually studied such as sensor damage, humidity effect and specificity. Moreover, this kind of sensor array produce huge amount of data, which also require to have a proper storage media and algorithms for extracting the information. Under these circumstances, conventional chemical sensor arrays do not provide enough performance for being directly introduced into the industry, hence new alternatives need to be developed and studied.

One alternative to the above explained is the Ion Mobility Spectrometry (IMS). IMS spectrometer is able to provide fast measurements to substances under ppm concentration. Furthermore, it is a portable instrument becoming really attractive to be used in situ applications. On the other hand, IMS has partial selectivity, its response is not lineal and low reproducibility. IMS have been mainly used for explosive, warfare or illicit drug detection (Eiceman et al., 1990, Eiceman, 1991, Eiceman and Stone, 2004). However, other fields have started to introduce IMS into these applications. For instance, in medicine there are specific IMS which has been designed for measurement the levels of propofol in anesthesia procedure(Zhou et al., 2012, Perl et al., 2009, Carstens et al., 2010). Nonetheless, there is not a deep algorithm development for enhancing and improving the performance of IMS spectra through signal processing.

Currently, signal processing in chemical sensors is not novel, because many algorithms have been designed to tackle specific problems in this kind of sensors. Nonetheless, in the IMS field, there has not been a deeply exploration for solving and enhancing IMS issues. Among them, alignment peaks, compound identification, non-linearity behavior, mixture effect, etc. Most of them are mainly present in bio-related application when hundreds of compounds are present and the main focus is to localize a specific subset of them. Therefore, the proper use or developments of new signal processing techniques are crucial for taking advantage of IMS.

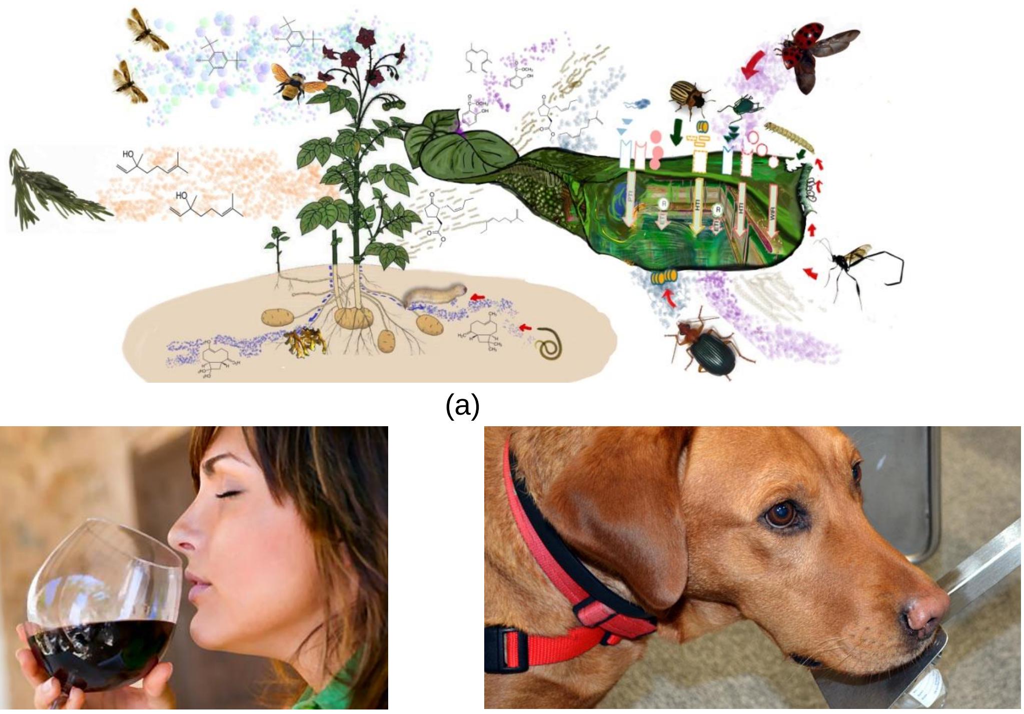
The main objectives in the present thesis are to use and develop signal processing techniques for tackling IMS open issues when samples from biomedical and food applications are studied - to simplify terminology we are going to refer as bio-related applications. Since IMS has been mainly used for explosive detection, which means usually a binary detection, bio-related applications provide complexity that is necessary to be explored by signal processing techniques. Thus, this thesis is divided into two main parts, the first one is devoted to provide IMS solutions for qualitative applications and enhancing the IMS spectra analysis through signal pre-processing algorithms, and the other one faces IMS problems in front of quantitative measurements. Before entering in the description of the different approaches, a brief explanation of the general scenario is needed. This chapter introduces this brief scenario description and explores the bio-related applications and methodology for volatile organic compounds (VOCs) analysis.

B. Volatile Organic Compounds (VOCs): An Overview

The olfactory system is one of the most important sense that nature have for survival proposes. Actually, the interaction between most of organisms and odors, which can be simplified as a stimulus, plays an important role in nature owing to help for communications, eating, mating, fighting and nesting. The olfactory system is also known as chemosensory sense due to convert chemical signals into perception. The mechanism of olfaction consist of sensing an external stimulus (chemical stimulus) and encoding this into an electric signal in neurons, then all signals are integrated and processed.

Figure A (a) shows how insects interact with plants and both organism help each other for survival purposes. Note that plant emits chemical substances which are received by insects. Thus, both, the fact that an insect can locate a host plant deflecting background of the surrounding media based on a pattern (Bruce et al., 2005, Runyon et al., 2006), or how domestic dog are able to detect certain human cancers by dog's sense of smell (MedicalDetectionDogs, 2014, Moser and McCulloch, 2010, Horvath et al., 2008, Leahy, 2004, Gordon et al., 2008) results quite interesting from a scientific and industrial point of view. Thus, mimicking some of these behaviors in our benefit using chemical sensors is feasible, challenging and surely useful.

Odors are a complex mixture of compounds and volatiles. According to the European Directive (1999/13/EC), volatile organic compound (VOC) is defined as any organic compound, which contain at least the element carbon and one or more of hydrogen, halogens, oxygen, having an initial boiling point of less than or equal to 20ºC measured at standard pressure of 0.01 kPa having a corresponding volatility under particular conditions of use. VOCs belong to different classes of chemical compounds including hydrocarbons, alcohols, aldehydes, ketones, acids, and esthers. Some authors differentiate VOCs that come from biological samples which are called as biological volatile organic compounds (Zhang and Li, 2010, Zhang et al., 2013). This difference helps to focus the goal of study in biological process and relate the compounds of interest only to a biological issue. In fact, Biological VOCs contains bioinformation related with biological metabolism and is noticable that every biological sample has a charctaristic fingerprint. In this cotext, the present tesis is refered only to the study of biological VOC.



(a)

(b) (c)

Figure A (a) Interaction between plants and insects (Villagra, 2014) (b) Wine testing (wineclube, 2010) (c)Detection of cancer based on dog's sniff (MedicalDetectionDogs, 2014)

VOCs are usually at very low concentrations (below ppm), and some of them cannot be detected by humans but animals and insects do. Actually, the odour perception of a human is much lower than a dog or mice, and the limit of detection varies from one aroma to another. In consequence, animals can be used for detecting some deases such as lung cancer (Figure A (c)) smelling odors than human being are not able to do it. However, some humans are able to smell some specific compounds for instance a person is able to reject adultaration wines based on the aroma (Figure A (b)). In the last years, there has been an interest about smell and technology. For instance, there are some news that exemplify this interest such as *bees is able to "sniff out" minute residues of explosives leading security agencies*(BBCnews, 2002), *breath test to diagnose infections* (BBCnews, 2013), body odor ID (ElMundo, 2014), or *corked wine confuse smell receptors* (ABCScience, 2013).

All of these considerations open new interesting research studies that are far from being trivial. Nonetheless, there is not too much comercial equipments in the market, if it is compared with other equipment biological inspired. Moreover, the analytical tool should also consider the huge amount of data that need to be proccesed in order to get reliable results. In fact, this field is still in development in order to create small, portable and unexpensive sensor or sensor arrays which would be able to provide relaible results. It must not be forgotten that is really attractive to have a non-invasive and rapid way of measurmenting samples than otherwise can be really unfeasible. Consequently, the interest in different research areas such as medicine, food industry, quality controls, security has been risen every year and important progress has been made up to day.

C. Volatile Organic Compounds (VOCs) Analysis

There are different areas where VOCs have been studied such as disease diagnosis, food safety, agriculture product quality control, warfare and explosive detection and drug illicit detection. The procedure for VOC analysis differs from application to application, but there are some generalized steps that can be followed in a broad way (Figure B). The whole process can be simplified in few steps: (i) sampling, (ii) analytical techniques to measurement samples and (iii) data analysis.

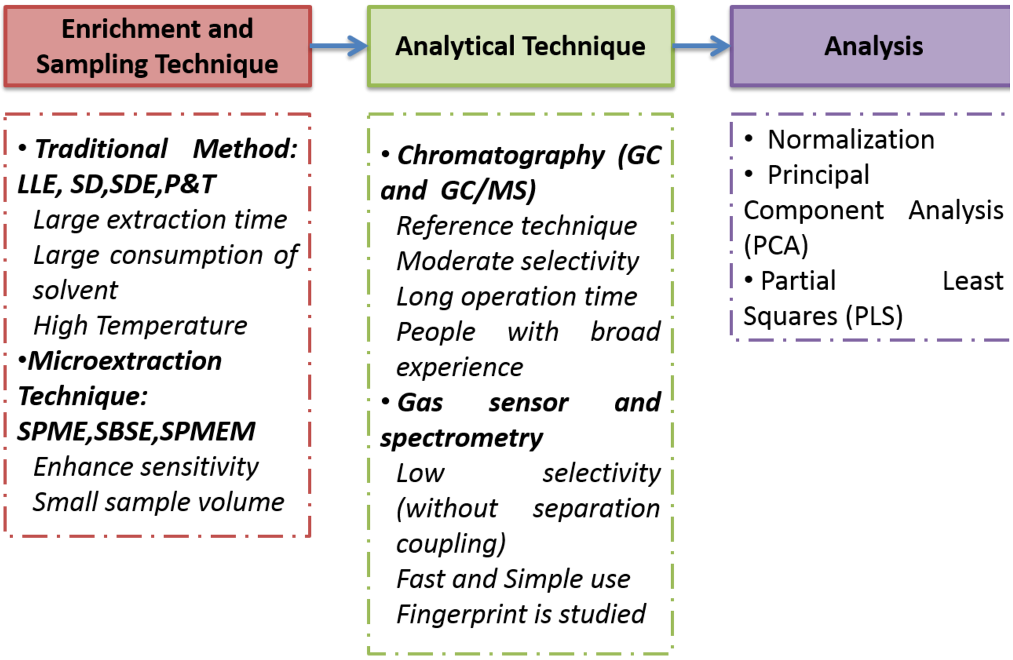


Figure B Schematic diagram summarizing the analysis of volatile organic compounds

i. Enrichment and Sampling technique

Biological systems produce complicate traces of VOCs whose chemicals differ in structure and physicochemical properties. Ideally, the main goal in sampling strategies is to get a good compromise between selectivity and sensitivity together with rejecting possible interferences or background. Nowadays, enrichment media have been deeply explored in this area for having excellent adsorption properties with high extraction capacity and selectivity. Thus, the development of nanometer-gas sensing materials, nano-gas adsorption material and metal-organic frameworks (MOFs) are the developing hotspot. Kida et al (Kida et al., 2010) has developed a new sensor of SnO2 nanocrystals that have a great selectivity and sensitivity to ethanol, formaldehyde and toluene at 5-400 ug ml-1 levels. Gu et al (Gu et al., 2012) has developed MOFs which have good discriminative properties for non-polar compounds for cancer detection. These examples show the potential use of enrichment media, but it is mainly focus in applications where the VOCs are really known and studied.

The traditional methods for sampling VOCs are liquid-liquid extraction (LLE) (Anthemidis and Ioannou, 2009, Pedersen-Bjergaard et al., 2000), steam distillation (SD) (Bowles and Apte, 1998, Fleck and Munro, 1965), simultaneous distillation extraction (SDE) (Zhang and Li, 2010), superficial fluid extraction (SFE) (Lang and Wai, 2001), purge and trap (P&T) (Pillonel et al., 2002, Contarini and Povolo, 2002) and microextraction techniques (Zhang and Li, 2010). SD and SDE are typically used for volatiles to be extracted from plants, and can cause unstable VOCs due to thermal decomposition. SFE has a high selectivity but is expensive if it is compared with other sampling techniques and it is mainly used for extract aroma VOCs from fruit or other plants. P&T are known as dynamic headspace in which VOC is continuously carried out through a purified inert gas and can be trapped in a sorbent such as Tenax. P&T is usually used for off-line sampling and can be coupled to other analytical techniques such as Gas Chromatography (GC). Microextraction techniques are miniaturized, rapid, simple and environmentally-friendly. The main advantage is that can be used in-vivo samples with a very small volume of headspace extraction phase. Solid Phase Microextraction (SPME) (Ouyang and Pawliszyn, 2006, Aulakh et al., 2005) is the most popular in VOCs analysis. SPME combines concentration, extraction and introduction in a unique step where the sensitivity is increased. Nowadays, new and challenging sampling techniques for biological sampling are molecularly imprinted polymers (MIPs) (Haupt, 2003) and nanoarrays (Lee et al., 2004). These novel techniques contribute in the analysis of specific chemical compounds improving the selectivity of the sensor. Certainty, nanoarrays also seeks to attach specific cells or proteins in bionalysis studies.

Certainly, beyond standard sampling techniques, specific sampling techniques have been developed owing to the diversity of biological scenarios. For instance, homemade sampling techniques have been developed for on-line measurements in breath analysis scenarios Figure C (Phillips et al., 2003a). In this case, the patient breathes out into the tube and the sample is following trapped into a sorbent trap to pre concentrate the sample. At the end the sample is analyzed using a conventional analytical technique such as gas chromatogram. The homemade sampling methods usually combine or adapt standard techniques to be able to fit in the application.

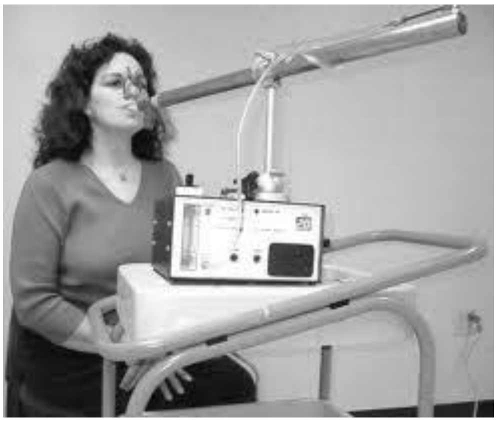


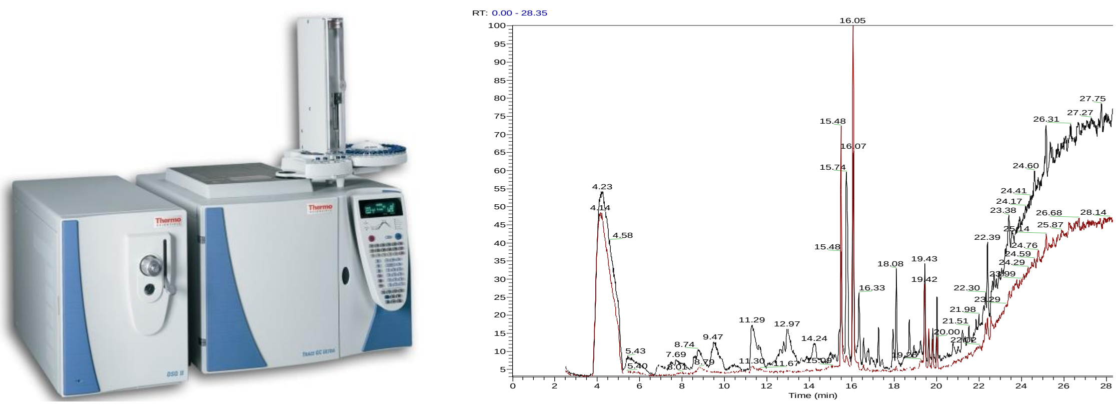
Figure C Breath sampling technique (Phillips et al., 2003a) developed by Michael Phillips to extract volatile organic compounds from human breath

ii. Analytical Techniques

There are few analytical techniques that have been used for volatile organic compounds analysis. The most common are liquid or gas chromatography due to this main characteristics that help in the understanding of complex applications. Nonetheless, needs of performing on-line measurements have motivated the use of new analytical techniques such as either electronic noses, or fast spectroscopic techniques. Certainty, there are advantages and disadvantages in the use of above analytical techniques from the technology to the subsequent data analysis which are explained bellow.

Gas chromatography and mass spectrometry (GC-MS) (Van den Velde et al., 2008, A et al., 2005) is the reference analytical technique in VOC analysis. GC-MS combine the separation of GC and the identification of the compounds given by MS (see Figure D (a)). The sample is introduced firstly in the GC in which the compounds flow through a capillary column and the compounds are retained depending on their physicochemical properties. A chromatogram (Figure D (b)) is generated in this initially step in which each compound can be represented by a peak at specific retention time. Then each compound is fragmented in the MS generating a specific fingerprint of the compound that is used for identification.

Since biological samples contain hundreds or thousands of VOCs, GC-MS provides more comprehensive results than other techniques. Consequently, GC/MS is well used as reference technique, especially when non target analysis is done. Additionally, GC/MS allows to be coupled to other sampling techniques, such as SPME or headspace, therefor sensitivity of the compounds can be improved and automatic analysis can be performed. Alternative, other configurations of GC, such as GCxGC(Mondello et al., 2008) or GC-MS-MS (Ternes et al., 2002), are found in the literature which seem to increase the selectivity or sensitivity, but very complex data is generated and advanced analytical tools are needed. On the other hand, the limitations of GC-MS are: the equipment is quite expensive, the time of analysis is high compared with other analytical techniques, is not a portable technique, and qualified personal is really needed.



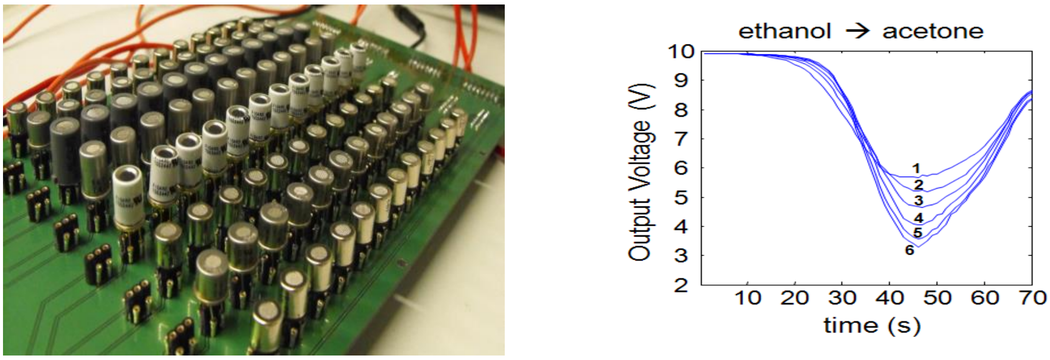
(a) (b)

Figure D (a) Commercial gas chromatography Mass Spectrometry (GC-MS) developed by Thermo Scientific (Scientific, 2014) (b) Two chromatograms from two different samples showing peaks at different retention time.

Electronic Noses (e-nose) attempt to emulate the human sense of smell intending to detect odors or flavors. E-nose combines chemical sensor arrays together with pattern recognition strategies in order to reproduce human olfaction process. An example of an electronic nose is depicted in Figure E (a) where several chemical sensors of two different manufactures are implemented (Gutierrez et al., 2011). This system attempts to emulate the redundancy and diversity that is found in olfactory systems. Besides home-made electronic noses, there are commercial e-noses in the market (Theenosecompany, 2015, SensigentIntelligent, 2015)

E-nose has partial selectivity and can discriminate a widespread of chemicals, but not every individual compound. The attractive aspect of e-nose is its small size and the ability to perform an on-line measurement in short time. Since the response of e-nose is based on changes of the electrical properties of the sensors to the odors, a computing system is need for enhancing interpretation of this technology. For instance, Figure E (b) shows a response of a chemical sensor array to changes from ethanol and acetone of a single sensor(Gutierrez et al., 2011). It can be seen that response of this particular sensor changes depending on the substance that is exposed.

Apart from the partial selectivity, the lack of reproducibility and reliability of conventional chemical sensors have hindered the presence of the electronic noses as a solution for industry applications. Moreover, e-nose are really sensitive with changes in temperature and humidity, therefore they use might be limited in some applications. In the last years, nanoarray-material-based sensors have been developed that attempt to improve the selectivity of e-nose. For instance, gold nanoparticles have been proposed by Peng et.al. (Peng et al., 2009) for rapidly discriminate the breath of lung cancer patients from healthy one in high humidity conditions. Other improvement is the use of protein receptors to attach specific odor molecules (Goldsmith et al., 2011).



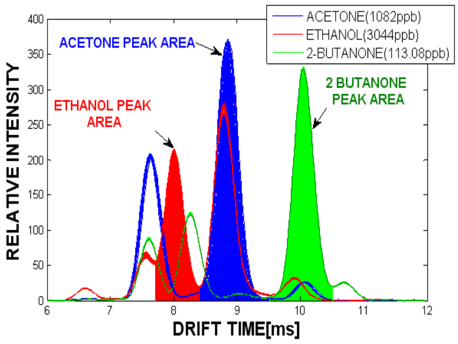
(a) (b)

Figure E (a) Chemical sensor arrays that are used for odor detection (b) response of the chemical array to changes of substances between ethanol and acetone. (Gutierrez et al., 2011)

Spectroscopic techniques are other alternative in VOCs analysis due to its potential use in real-time analysis. Spectroscopic techniques allow a fast analysis with a moderate sensitive, but lower selectivity without any pre-separation technique coupled to it. Among of them, infrared spectroscopy (IR) (Elosua et al., 2009), ultraviolet-visible spectroscopy (UV-Vis) (Yoon et al., 2007)and ion mobility spectroscopy (IMS) are the most frequently used (Eiceman et al., 2014).

IMS is based on ionization of gas-phase molecules. This ionization generates new ionized molecules that travel into a drift tube region where an electric field is applied. The electric field accelerates the incoming molecules in such a way that different mobilities are produced from different molecules. The molecule mobility will depend on the size, wide and other physic and chemical properties. At the end of the IMS a detector is located, thus a spectra is generated in which each peak represents the ionic species generated in the measurement process. (see Figure F (b)). Nowadays, the use of IMS has been incremented due to small size and its fast response which is attractive in real-time applications. Moreover, there are some IMS that are portables and some of them provide other kind of sensors improving the substance identification capability such as GDA2 developed by Airsense, Germany (see Figure F (a)). The response of the IMS can be interpretable because a peak in the spectra should be correlated with a molecule. Nonetheless, two different molecules might have closer peaks that sometimes make difficult its interpretation. In Figure F (b) the spectra of ethanol and acetone are quite similar. Note that ethanol have a peak which is too close to acetone peak. In order to solve these problems, signal processing techniques are required to enhance the sensitivity or selectivity of IMS. In chapter one a deeper explanation is found.

(a)



(b)

Figure F (a) commercial Ion mobility spectrometer GDA2 Airsense, Germany (b) typical response of IMS in which three different substances was separately measured.

iii. Signal Analysis

The first issue to be considered is to enhance the signal (chromatogram, spectra, response) using pre-processing strategies whose goal is to eliminate artifacts, noise reduction, peak detection and normalization. Certainly, the pre-processing steps are totally linked to the kind of signal obtained by the analytical technique. For instance, baseline correction is critical in chromatograms to ensure a properly peak detection above the noise level of the instrument, or alignment is also critical to ensure that a compound is the same in different samples both GC/MS and IMS analysis.

Besides preprocessing, there are a huge amount of possibilities for signal analysis. Which is the best way to face the work depends on the application. On the other hand, most of the analytical signal processing needs can be classified in two main groups: qualitative/classification analysis or quantitative/regression analysis. For example, principal component analysis (PCA) is an extensively used tool for visualizing the data that can be associated to qualitative analysis, and to solve a calibration problem falls into the field of quantitative analysis.

In this work I will present solutions for VOC analysis based on IMS measurements. The primarily use of IMS was in explosive and illicit chemicals detection in which the detection was based on a binary decision using one or few known compounds. It is only a few years ago that the interest of using multivariate techniques to address complex data from IMS have been risen, in virtue of novel bio-related applications has appeared too. These bio-related applications contain thousands of compounds which makes unfeasible the use of univariate strategies because the behavior of the compounds into IMS brings additional drawbacks. For example overlapping of peaks from unknown compounds is a problem to be solved. In the same context, quantitative and qualitative problems have been poorly explored. Indeed, quantitative problems are better solved than qualitative ones. Univariate calibration is usually used for solving qualitative problems, which means to choose a relevant peak (compound) and fit a curve. On the other hand, visual inspection is used for solving qualitative problems. Therefore, multivariate strategies are proposed to handle with these "new" problems for getting reliable results.

D. Biomedical and biological application using VOCs analysis

Different scenario have been addressed in biological VOCs analysis among of them food safety, clinical studies, insect's behavior, and quality control.

For instance, in clinical studies one of the main focuses is to find a set of biomarkers related to some specific pathology such as lung cancer using a reliable non-invasive and early diagnosis tool. The underlying idea is that there might be specific subsets of VOCs that should be linked to a specific pathology. The goal is to extract specific information from hundreds or thousands of compounds that can be found in breath, urine or skin samples. In this context, to choose a sampling and analytical technique are critical but also the data analysis for finding the specific biomarkers and rejecting the non-informative compounds, is a key point. GC/MS is the gold standard for finding biomarkers in such complex matrix, but its lack of portability is sometimes a limitation for being used in hospitals as point of care system. Thus e-nose and spectrometry, especially ion mobility spectrometry, emerge as a complementary alternative for in-situ analysis. Lung cancer (Westhoff et al., 2009, Phillips et al., 2007, Phillips et al., 2003a, Di Natale et al., 2003), breast cancer(Phillips et al., 2010, Phillips et al., 2003b) ,tuberculosis and asthma(Delfino et al., 2003) are some examples of clinical studies using biological VOCs analysis.

The other big area of study is related to quality control in food and beverages. This area pays attention in enhance quality control or improve industrial process to reject spoiling food. For example, there are studies to monitor freshness of fruit (Lindinger et al., 1998, Borsdorf et al., 2009), meat (Vestergaard et al., 2007, Vestergaard et al., 2006, Karpas et al., 2002, Bota and Harrington, 2006), fish (Karpas et al., 2002), etc. The fraud detection it also studied for wine industry (Holmberg, 2010, Garrido-Delgado et al., 2011a), Iberian ham(Alonso et al., 2008), olive oil (Garrido-Delgado et al., 2011b), etc. Also, detection of contaminants in final products has risen in the last years because it have a direct economic effect in the industry, which might be caused by both environmental and manufacturing factors such as detection of TCA in wine and cork stoppers (Maggi et al., 2008, Karpas et al., 2012). Another hotspot is the screening and detection of pesticides in plants (Borsdorf et al., 2009)or infections in plants (Schmelz et al., 2003, Runyon et al., 2006, Lang and Wai, 2001, Cardoza et al., 2002).

E. Summary

During this chapter the importance of Volatile Organic Compounds (VOCs) has been discussed and the diverse applications where VOCs are used, among of them clinical, food control and safety studies. The biological samples, which are formed by hundreds of VOCs, have specific and relevant information that need to be extracted for being used in the different areas of study. However, it is really important to pay attention in how to obtain this information and perform the subsequent analysis in order to get reliable and accurate results.

Furthermore, there have been introduced the typical procedure for VOCs analysis. Obviously and depending on the application there are several ways for sampling and extract VOCs from the sample. Furthermore, there are different analytical techniques to be used with advantages and disadvantages. One of these instruments is IMS providing fast, simple and in-situ measurements. IMS is an attractive alternative analytical technique for VOCs analysis, especially in applications where a fast analysis is required. On the other hand, IMS has a moderate selectivity and non-linear behave. Nevertheless, using the proper signal processing algorithms a considerable improvement can be obtained in spectra analysis of IMS. Chapter One introduces the pros and cons of IMS used as stand-alone instrument and the potential use in bio-related applications. Moreover, an extended summary will be presented covering univariate and multivariate strategies together with their use in IMS nowadays in chapter two. In the present thesis, specific data sets were created in order to test different signal processing strategies that is presented in chapter three. The results are present in chapter four for qualitative response and chapter five for quantitative results using univariate and multivariate strategies for bio-related applications. Finally, the main conclusions are presented at the end of this thesis.

F. References

A, J., Trygg, J., Gullberg, J., Johansson, A. I., Jonsson, P., Antti, H., Marklund, S. L. & Moritz, T. 2005. Extraction and GC/MS Analysis of the Human Blood Plasma Metabolome. *Analytical Chemistry,* 77**,** 8086-8094.

ABCScience. 2013. ***Corked wine confuses smell receptors*** [Online]. Available: http://www.abc.net.au/science/articles/2013/09/17/3850029.htm 2014].

Alonso, R., Rodriguez-Estevez, V., Dominguez-Vidal, A., Ayora-Canada, M. J., Arce, L. & Valcarel, M. 2008. Ion mobility spectrometry of volatile compounds from Iberian pig fat for fast feeding regime authentication. *Talanta,* 76**,** 591-596.

Anthemidis, A. N. & Ioannou, K.-I. G. 2009. Recent developments in homogeneous and dispersive liquid–liquid extraction for inorganic elements determination. A review. *Talanta,* 80**,** 413-421.

Aulakh, J. S., Malik, A. K., Kaur, V. & Schmitt-Kopplin, P. 2005. A Review on Solid Phase Micro Extraction—High Performance Liquid Chromatography (SPME-HPLC) Analysis of Pesticides. *Critical Reviews in Analytical Chemistry,* 35**,** 71-85.

Baumbach, J. I., Vautz, W., Ruzsanyi, V. & Freitag, L. 2005. Metabolites in human breath: Ion mobility spectrometers as diagnostic tools for lung diseases. *Breath Analysis: for Clinical Diagnosis and ATherapeutic Monitoring***,** 53-66.

BBCnews. 2002. *B**ees to 'sniff out' explosives*** [Online]. 2014].

BBCnews. 2013. *Lung infection identified using 'breath-print'* [Online]. Available: www.bbc.co.uk/news/health-20975948 2014].

Borsdorf, H., Roetering, S., Nazarov, E. & Weickhardt, C. 2009. Rapid screening of pesticides from fruit surfaces: Preliminary examinations using a laser desorption-differential mobility spectrometry coupling. *International Journal of Ion Mobility Spectrometry,* 12**,** 15-22.

Bota, G. M. & Harrington, P. B. 2006. Direct detection of trimethylamine in meat food products using ion mobility spectrometry. *Talanta,* 68**,** 629-635.

Bowles, K. C. & Apte, S. C. 1998. Determination of Methylmercury in Natural Water Samples by Steam Distillation and Gas Chromatography−Atomic Fluorescence Spectrometry. *Analytical Chemistry,* 70**,** 395-399.

Bruce, T. J. A., Wadhams, L. J. & Woodcock, C. M. 2005. Insect host location: a volatile situation. *Trends in Plant Science,* 10**,** 269-274.

Buszewski, B., Kęsy, M., Ligor, T. & Amann, A. 2007. Human exhaled air analytics: biomarkers of diseases. *Biomedical Chromatography,* 21**,** 553-566.

Cardoza, Y. J., Alborn, H. T. & Tumlinson, J. H. 2002. In vivo volatile emissions from peanut plants induced by simultaneous fungal infection and insect damage. *Journal of Chemical Ecology,* 28**,** 161-174.

Carstens, E., Hirn, A., Quintel, M., Nolte, J., Jünger, M., Perl, T. & Vautz, W. 2010. On-line determination of serum propofol concentrations by expired air analysis. *International Journal for Ion Mobility Spectrometry,* 13**,** 37-40.

Contarini, G. & Povolo, M. 2002. Volatile Fraction of Milk: Comparison between Purge and Trap and Solid Phase Microextraction Techniques. *Journal of Agricultural and Food Chemistry,* 50**,** 7350-7355.

Delfino, R. J., Gong, H., Linn, W. S., Hu, Y. & Pellizzari, E. D. 2003. Respiratory symptoms and peak expiratory flow in children with asthma in relation to volatile organic compounds in exhaled breath and ambient air. *Journal of Exposure Analysis and Environmental Epidemiology,* 13**,** 348-363.

Di Natale, C., Macagnano, A., Martinelli, E., Paolesse, R., D'Arcangelo, G., Roscioni, C., Finazzi-Agro, A. & D'Amico, A. 2003. Lung cancer identification by the analysis of breath by means of an array of non-selective gas sensors. *Biosensors & Bioelectronics,* 18**,** 1209-1218.

Dinatale, C., Damico, A. & Davide, F. A. M. 1993. REDUNDANCY IN SENSOR ARRAYS. *Sensors and Actuators a-Physical,* 37-8**,** 612-617.

Eiceman, G. A. 1991. ADVANCES IN ION MOBILITY SPECTROMETRY - 1980-1990. *Critical Reviews in Analytical Chemistry,* 22**,** 17-36.

Eiceman, G. A., Blyth, D. A., Shoff, D. B. & Snyder, A. P. 1990. SCREENING OF SOLID COMMERCIAL PHARMACEUTICALS USING ION MOBILITY SPECTROMETRY. *Analytical Chemistry,* 62**,** 1374-1379.

Eiceman, G. A., Karpas, Z. & Hill, H. H. J. 2014. *Ion Mobility Spectrometry,* 6000 Broken Sound Parkway NW, Suite 300.

Eiceman, G. A. & Stone, J. A. 2004. Ion mobility spectrometers in national defense. *Analytical Chemistry,* 76**,** 390A-397A.

ElMundo. 2014. *Tecnología 'made in Spain' para identificar personas por su olor* [Online]. Available: http://www.elmundo.es/ciencia/2014/02/06/52f2411c268e3ef3738b456e.html 2014].

Elosua, C., Bariain, C., Matias, I. R., Arregui, F. J., Vergara, E. & Laguna, M. 2009. Optical fiber sensing devices based on organic vapor indicators towards sensor array implementation. *Sensors and Actuators B: Chemical,* 137**,** 139-146.

Fernandez, L., Gutierrez-Galvez, A. & Marco, S. 2010. Gas sensor array system inspired on the sensory diversity and redundancy of the olfactory epithelium. *Eurosensors Xxiv Conference,* 5**,** 25-28.

Firestein, S. 2001. How the olfactory system makes sense of scents. *Nature,* 413**,** 211-218.

Fleck, A. & Munro, H. N. 1965. The determination of organic nitrogen in biological materials: A review. *Clinica Chimica Acta,* 11**,** 2-12.

Fonollosa, J., Fernandez, L., Huerta, R., Gutierrez-Galvez, A. & Marco, S. 2013. Temperature optimization of metal oxide sensor arrays using Mutual Information. *Sensors and Actuators B-Chemical,* 187**,** 331-339.

Furton, K. G. & Myers, L. J. 2001. The scientific foundation and efficacy of the use of canines as chemical detectors for explosives. *Talanta,* 54**,** 487-500.

Garrido-Delgado, R., Arce, L., Guaman, A. V., Pardo, A., Marco, S. & Valcarcel, M. 2011a. Direct coupling of a gas-liquid separator to an ion mobility spectrometer for the classification of different white wines using chemometrics tools. *Talanta,* 84**,** 471-479.

Garrido-Delgado, R., Mercader-Trejo, F., Arce, L. & Valcarcel, M. 2011b. Enhancing sensitivity and selectivity in the determination of aldehydes in olive oil by use of a Tenax TA trap coupled to a UV-ion mobility spectrometer. *Journal of Chromatography A,* 1218**,** 7543-7549.

Goldsmith, B. R., Mitala, J. J., Josue, J., Castro, A., Lerner, M. B., Bayburt, T. H., Khamis, S. M., Jones, R. A., Brand, J. G., Sligar, S. G., Luetje, C. W., Gelperin, A., Rhodes, P. A., Discher, B. M. & Johnson, A. T. C. 2011. Biomimetic Chemical Sensors Using Nanoelectronic Readout of Olfactory Receptor Proteins. *ACS Nano,* 5**,** 5408-5416.

Gordon, R. T., Schatz, C. B., Myers, L. J., Kosty, M., Gonczy, C., Kroener, J., Tran, M., Kurtzhals, P., Heath, S., Koziol, J. A., Arthur, N., Gabriel, M., Hemping, J., Hemping, G., Nesbitt, S., Tucker-Clark, L. & Zaayer, J. 2008. The use of canines in the detection of human cancers. *Journal of Alternative and Complementary Medicine,* 14**,** 61-67.

Gu, Z.-Y., Yang, C.-X., Chang, N. & Yan, X.-P. 2012. Metal-Organic Frameworks for Analytical Chemistry: From Sample Collection to Chromatographic Separation. *Accounts of Chemical Research,* 45**,** 734-745.

Gutierrez, A., Fernandez, L. & Marco, S. 2011. Study of sensor diversity and redundancy for chemical mixtures. *International Symposium on Olfaction and Electronic Nose.* Rockefeller University, New York City.

Haupt, K. 2003. Peer Reviewed: Molecularly Imprinted Polymers: The Next Generation. *Analytical Chemistry,* 75**,** 376 A-383 A.

Holmberg, L. 2010. Wine Fraud. *International Journal of Wine Research,* 2**,** 105–113.

Horvath, G., Jarverud, G. a. K., Jarverud, S. & Horvath, I. 2008. Human ovarian carcinomas detected by specific odor. *Integrative Cancer Therapies,* 7**,** 76-80.

Horvath, I., Lazar, Z., Gyulai, N., Kollai, M. & Losonczy, G. 2009. Exhaled biomarkers in lung cancer. *European Respiratory Journal,* 34**,** 261-275.

Karpas, Z., Guaman, A. V., Calvo, D., Pardo, A. & Marco, S. 2012. The potential of ion mobility spectrometry (IMS) for detection of 2,4,6-trichloroanisole (2,4,6-TCA) in wine. *Talanta,* 93**,** 200-205.

Karpas, Z., Tilman, B., Gdalevsky, R. & Lorber, A. 2002. Determination of volatile biogenic amines in muscle food products by ion mobility spectrometry. *Analytica Chimica Acta,* 463**,** 155-163.

Kida, T., Doi, T. & Shimanoe, K. 2010. Synthesis of monodispersed SnO2 nanocrystals and their remarkably high sensitivity to volatile organic compounds. *Chemistry of Materials,* 22**,** 2662-2667.

Lang, Q. & Wai, C. M. 2001. Supercritical fluid extraction in herbal and natural product studies — a practical review. *Talanta,* 53**,** 771-782.

Leahy, M. 2004. Olfactory detection of human bladder cancer by dogs - Cause or association? *British Medical Journal,* 329**,** 1286-1286.

Lee, K.-B., Kim, E.-Y., Mirkin, C. A. & Wolinsky, S. M. 2004. The Use of Nanoarrays for Highly Sensitive and Selective Detection of Human Immunodeficiency Virus Type 1 in Plasma. *Nano Letters,* 4**,** 1869-1872.

Lindinger, W., Hansel, A. & Jordan, A. 1998. Proton-transfer-reaction mass spectrometry (PTR-MS): on-line monitoring of volatile organic compounds at pptv levels. *Chemical Society Reviews,* 27**,** 347-354.

Maggi, L., Mazzoleni, V., Fumi, M. D. & Salinas, M. R. 2008. Study of 2,4,6-trichloroanisole production by fungi isolated from cork and grapes and grown in presence of 2,4,6-trichlorophenol. *Industrie delle Bevande,* 37**,** 342-345.

MedicalDetectionDogs. 2014. *Medical Detection Dogs* [Online]. Available: http://medicaldetectiondogs.org.uk/index.html [Accessed 2014 2014].

Mondello, L., Tranchida, P. Q., Dugo, P. & Dugo, G. 2008. Comprehensive two-dimensional gas chromatography-mass spectrometry: A review. *Mass Spectrometry Reviews,* 27**,** 101-124.

Moser, E. & McCulloch, M. 2010. Canine scent detection of human cancers: A review of methods and accuracy. *Journal of Veterinary Behavior-Clinical Applications and Research,* 5**,** 145-152.

Oh, Y., Lee, Y., Heath, J. & Kim, M. 2015. Applications of Animal Biosensors: A Review. *Ieee Sensors Journal,* 15**,** 637-645.

Ouyang, G. & Pawliszyn, J. 2006. Recent developments in SPME for on-site analysis and monitoring. *TrAC Trends in Analytical Chemistry,* 25**,** 692-703.

Pauling, L., Robinson, A. B., Teranish.R & Cary, P. 1971. QUANTITATIVE ANALYSIS OF URINE VAPOR AND BREATH BY GAS-LIQUID PARTITION CHROMATOGRAPHY. *Proceedings of the National Academy of Sciences of the United States of America,* 68**,** 2374-&.

Pedersen-Bjergaard, S., Rasmussen, K. E. & Grønhaug Halvorsen, T. 2000. Liquid–liquid extraction procedures for sample enrichment in capillary zone electrophoresis. *Journal of Chromatography A,* 902**,** 91-105.

Pedro Santos, J., Lozano, J., Aleixandre, M., Arroyo, T., Mariano Cabellos, J., Gil, M. & del Carmen Horrillo, M. 2010. Threshold detection of aromatic compounds in wine with an electronic nose and a human sensory panel. *Talanta,* 80**,** 1899-1906.

Peng, G., Tisch, U., Adams, O., Hakim, M., Shehada, N., Broza, Y. Y., Billan, S., Abdah-Bortnyak, R., Kuten, A. & Haick, H. 2009. Diagnosing lung cancer in exhaled breath using gold nanoparticles. *Nat Nano,* 4**,** 669-673.

Perl, T., Carstens, E., Hirn, A., Quintel, M., Vautz, W., Nolte, J. & Junger, M. 2009. Determination of serum propofol concentrations by breath analysis using ion mobility spectrometry. *British Journal of Anaesthesia,* 103**,** 822-827.

Persaud, K. & Dodd, G. 1982. ANALYSIS OF DISCRIMINATION MECHANISMS IN THE MAMMALIAN OLFACTORY SYSTEM USING A MODEL NOSE. *Nature,* 299**,** 352-355.

Phillips, M., Altorki, N., Austin, J. H. M., Cameron, R. B., Cataneo, R. N., Greenberg, J., Kloss, R., Maxfield, R. A., Munawar, M. I., Pass, H. I., Rashid, A., Rom, W. N. & Schmitt, P. 2007. Prediction of lung cancer using volatile biomarkers in breath. *Cancer Biomarkers,* 3**,** 95-109.

Phillips, M., Cataneo, R. N., Cummin, A. R. C., Gagliardi, A. J., Gleeson, K., Greenberg, J., Maxfield, R. A. & Rom, W. N. 2003a. DEtection of lung cancer with volatile markers in the breath*. *Chest,* 123**,** 2115-2123.

Phillips, M., Cataneo, R. N., Ditkoff, B. A., Fisher, P., Greenberg, J., Gunawardena, R., Kwon, C. S., Rahbari-Oskoui, F. & Wong, C. 2003b. Volatile Markers of Breast Cancer in the Breath. *The Breast Journal,* 9**,** 184-191.

Phillips, M., Cataneo, R. N., Saunders, C., Hope, P., Schmitt, P. & Wai, J. 2010. Volatile biomarkers in the breath of women with breast cancer. *Journal of Breath Research,* 4**,** 8.

Phillips, M., Herrera, J., Krishnan, S., Zain, M., Greenberg, J. & Cataneo, R. N. 1999. Variation in volatile organic compounds in the breath of normal humans. *Journal of Chromatography B,* 729**,** 75-88.

Pillonel, L., Bosset, J. O. & Tabacchi, R. 2002. Rapid Preconcentration and Enrichment Techniques for the Analysis of Food Volatile. A Review. *LWT - Food Science and Technology,* 35**,** 1-14.

Runyon, J. B., Mescher, M. C. & De Moraes, C. M. 2006. Volatile chemical cues guide host location and host selection by parasitic plants. *Science,* 313**,** 1964-1967.
Schmelz, E. A., Engelberth, J., Alborn, H. T., O'Donnell, P., Sammons, M., Toshima, H. & Tumlinson, J. H. 2003. Simultaneous analysis of phytohormones, phytotoxins, and volatile organic compounds in plants. *Proceedings of the National Academy of Sciences of the United States of America,* 100**,** 10552-10557.
Scientific, T. 2014. *Gas Chromatogram Mass Spectrometry* [Online]. Available: www.thermo.fr.
SensigentIntelligent, s. 2015. *Sensigent Intelligent sensing solutions* [Online]. Available: http://www.sensigent.com/products/cyranose.html [Accessed 2015 2015].
Teranish.R, Robinson, A. B., Cary, P., Mon, T. R. & Pauling, L. 1972. GAS-CHROMATOGRAPHY OF VOLATILES FROM BREATH AND URINE. *Analytical Chemistry,* 44**,** 18-&.
Ternes, T. A., Andersen, H., Gilberg, D. & Bonerz, M. 2002. Determination of Estrogens in Sludge and Sediments by Liquid Extraction and GC/MS/MS. *Analytical Chemistry,* 74**,** 3498-3504.
Theenosecompany. 2015. *The enose company* [Online]. Available: http://www.enose.nl/.
Van den Velde, S., Nevens, F., Van Hee, P., van Steenberghe, D. & Quirynen, M. 2008. GC-MS analysis of breath odor compounds in liver patients. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences,* 875**,** 344-348.
Vestergaard, J. S., Haugen, J.-E. & Byrne, D. V. 2006. Application of an electronic nose for measurements of boar taint in entire male pigs. *Meat Science,* 74**,** 564-577.
Vestergaard, J. S., Martens, M. & Turkki, P. 2007. Analysis of sensory quality changes during storage of a modified atmosphere packaged meat product (pizza topping) by an electronic nose system. *Lwt-Food Science and Technology,* 40**,** 1083-1094.
Villagra, C. 2014. *Sensory Ecology of Plant-Animal Interaction Lab* [Online]. Available: cavillagra.files.wordpress.com [Accessed 2014 2014].
Westhoff, M., Litterst, P., Freitag, L., Urfer, W., Bader, S. & Baumbach, J. I. 2009. Ion mobility spectrometry for the detection of volatile organic compounds in exhaled breath of patients with lung cancer: results of a pilot study. *Thorax,* 64**,** 744-748.
wineclube. 2010. *How to Conduct Comparative and Blind Wine Tastings* [Online]. Available: http://www.wineclub.org/2010/10/comparative-blind-wine-tastings/.
Yoon, J., Chae, S. K. & Kim, J.-M. 2007. Colorimetric Sensors for Volatile Organic Compounds (VOCs) Based on Conjugated Polymer-Embedded Electrospun Fibers. *Journal of the American Chemical Society,* 129**,** 3038-3039.
Zhang, Z. M. & Li, G. K. 2010. A review of advances and new developments in the analysis of biological volatile organic compounds. *Microchemical Journal,* 95**,** 127-139.
Zhang, Z. M., Ma, Y. J. & Li, G. K. 2013. Progress on the analytical methodology for biological volatile organic compounds. *Analytical Methods,* 5**,** 20-29.
Zhou, Q., Wang, W., Cang, H., Du, Y., Han, F., Chen, C., Cheng, S., Li, J. & Li, H. 2012. On-line measurement of propofol using membrane inlet ion mobility spectrometer. *Talanta,* 98**,** 241-246.